have a matched related donor. While transplant outcomes with HLA matched unrelated donors continue to improve, this requires stringent HLA matching which puts ethnic minorities at a disadvantage. In this scenario, umbilical cord blood (UCB) has proven to be a valuable alternative source of hematopoietic stem cells as it allows for successful engraftment and decreased GVHD for a greater degree of HLA disparity. This is of particular importance for the Hispanic and African American populations in the United States who often receive ethnically mismatched products due to low representation in the donor pool despite increased recruitment efforts.

The St. Louis Cord Blood Bank (SLCBB) retrospectively evaluated outcomes for 727 patients based on recipient/donor ethnic match. 543 (74.7%) patients were matched and 184 (25.3%) were not ethnically matched. Ninety two percent of all patients received singleton cord blood transplant and 8 percent received dual cord blood transplant with both products shipped from the SLCBB. Patient and product characteristics are presented in Table 1.

There were no differences between the groups with respect to exposure to TBI, TNC dose/kg, CD34 dose/kg, and CFU dose/kg. There was also no correlation between groups with respect to incidence and severity of acute and chronic graft versus host disease ($p>.05$). Although the ethnically matched group had a higher degree of HLA match ($p<.05$) time to neutrophil recovery was not impacted ($p>.05$; median days matched = 22, median days unmatched = 20). Survival did not differ between the groups at 100 day and $\geq$ 1 year time points ($p>.05$).

This analysis supports the continued use of UCB as a source of hematopoietic reconstitution for all populations.

## 405

### IV BUSULFAN BASED CONDITIONING REGIMEN FOR HAPLOIDENTICAL TRANSPLANTATION

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**Introduction:** Haploidentical transplant (HAPLO) offers the opportunity for a cure to those patients without any other available donor. Following Luznik L et al experience (BBMT 2008; 14:641-650) with a non-myeloablative schema where relapses were the main issue, we developed our own conditioning model using IV busulfan (3,2 mg/kg on day -3) instead of 200cGy TBI, while maintaining post-transplant high dose cyclophosphamide for GVHD prophylaxis on days +3 and +4 followed by cyclosporine and MMF. Due to encouraging results with our initial experience, IV busulfan was increased to 3,2 mg/kg/day for 2 days in order to reduce relapse incidence and not to increase transplant related morbidity and mortality (TRM).

**Patients and Methods:** From Dec-2007, 12 patients (9 males, median age 39y range 19-55) have received an HAPLO at our institution for the treatment of AML (3), ALL (1), MDS (1) Myeloma (2) or Hodgkin's disease (5), all of them in advanced phases of their diseases. The conditioning regimen consisted of Fludarabine 30 mg/m2 for 5 days, Cyclophosphamide 14,5 mg/kg for 2 days and IV Busulfan 3,2 mg/kg for 1 day was used in 7 patients and 3,2 mg/kg for 2 days in the last 5. Bone marrow was the source of progenitors in 10 and GCSF mobilized peripheral blood without T-depletion in 2. Eight had received a previous autologous (6) or umbilical cord blood transplant (2, 1 after graft failure).

**Results:** Median follow up is 12.5 months (0.5-31mo). All patients engrafted properly. Toxicities of the conditioning regimen included grade1-2 mucositis (9) and infections (febrile neutropenia and CMV reactivations in 11, and 1 Pseudomona vulvar cellulitis). Only 1 patient died from toxicity before day +100 (VOD/MOF at +15d, refractory ALL). Acute GVHD grade II-IV affected to 4/11 patients, being grade III-IV in 2. Only 2 out of 8 cases at risk developed chronic GVHD that was extensive in 1. After a median time of 6.5 mo (2.5-30 mo), 7 patients are in continuous remission and 2 patients are alive in relapse. Two patients died beyond 1 year: 1 progressive multifocal leukoencephalopaty at +14 mo and relapse and progression of AML at +19 mo, respectively.

**Conclusions:** In our limited experience, IV Busulfan was well tolerated as part of the conditioning regimen for HAPLO. A potential reduction in relapse incidence without an increase in TRM would be obtained by this approach. HAPLO is a good chance for rescueing patients in graft failure after cord blood transplant.

## 406

### REAL-TIME QUANTITATIVE-PCR (qrt-PCR) USING AlleleSEQR TECHNOLOGY: TO ASSESS CHIMERISM IN PEDIATRICS PATIENTS UNDERGOING HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT)

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Post-transplantation chimerism monitoring in HSCT is pivotal to assess engraftment, rejection or relapse. There has been previous studies performed using qrt-PCR to detect chimerism by different technologies. Our purpose is to describe qrt-PCR as a sensitive and reproducible method to assess chimerism using AlleleSEQR (Cellera). A total of 112 peripheral blood samples were obtained from 43 allogeneic HSCT. qrt-PCR (Applied Biosystems 7500) was performed using 34 alleles that are designed to be a bi-allelic insertion/deletion (indel) polymorphism in the human genome. Extraction of DNA was performed using QIAamp DNA Blood Mini Kit (Qiagen) of both donor and recipient cells pre transplant (n = 43) then were screened for their informative alleles. At least 2 alleles were chosen that amplified with a $C_T$ value less than 34.0, but greater than or equal to 30.0 with a $\Delta C_T$ of -2.0 to +2.0. Post transplant samples (n = 69) were then separated in three subsets: Total WBC using the Erythrocyte Lysis Buffer (Qiagen), T-cell and Myeloid cells using RosetteSep™ T-cell and Myeloid Enrichment kits (Stem Cell Technologies) followed by DNA extraction and quantitation using the chosen informative alleles. To determine the sensitivity a serial dilution curve is shown in Table 1 with a r = 1. Reproducibility was validated by a reference lab using 18 unknown samples with a correlation of r = 0.98. Subset analysis was reported as percent donor total: 91.9 ± 2.22, median 99.6 (35.5-100), T-cell: 93.7 ± 2.56, median 99.6 (29.6-100), myeloid: 91.2 ± 3.23, median 99.9 (9.30-100) with a p value of < 0.0001. Results indicate that the use of this technology is reproducible, sensitive and accurate to assess donor Chimerism in pediatric patients undergoing HSCT.

**Table 1. qrt-PCR Sensitivity Determination**

| Serial Dilution | qrt-PCR Results |
|---|---|
| 0.1% | 0.086% |
| 0.2% | 0.16% |
| 1% | 1.0% |
| 2% | 1.9% |
| 10% | 9.2% |

## 407

### RAPID IDENTIFICATION OF INFORMATIVE MARKERS FROM MULTIPLE DONORS FOR STEM CELL TRANSPLANT ENGRAFTMENT MONITORING

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We have designed a system of RUO qPCR assays and software enabling highly sensitive, stem cell engraftment monitoring. Our approach allows for facile quantification of minute amounts of minor components in a mixture, while also reducing workflow and analysis burdens. This approach expands the linear dynamic range over which increasing mixed chimerism may be monitored, effectively increasing the probability of detecting adverse transplant events earlier.

We utilize a panel of 34 qPCR research assays to bi-allelic indels across the genome. Our system affords rapid pre-transplant marker identification and quantification of recipient levels post-transplant. It enables marker identification and quantification to occur within a few hours and without the need for capillary electrophoresis. In an effort to decrease our current sample volume requirements, increase throughput and enable multiple donor screening scenarios, we evaluated a multiplexed assay approach for marker identification.

The qPCR assays are multiplexed, with 2-3 assays per well. The probes are labeled with FAM-BHQ1, California Orange-BHQ1 and Quasar 670-BHQ2. The presence of alleles is scored in relation to amplification of an endogenous control assay. The results of samples within a group are compared against each other to identify informative markers for each sample. Up to eight samples can be

evaluated in a single 96-well plate, with a variable number and configuration of samples within a group.
**Results:** The described multiplexed qPCR approach yielded results which were comparable to those generated when using the assays individually. When the single assay algorithms were applied to the samples tested in the multiplexed format, the same genotyping calls were made and informative alleles identified.
**Conclusions:** Use of this RUO, multiplexed qPCR system for screening of multiple specimens enabled rapid identification of informative markers for all samples without post-PCR processing, while dramatically decreasing sample volume requirements.

## 408

### DONOR AND RECIPIENT HBV IMMUNITY BEFORE TRANSPLANTATION SIMULTANEOUSLY AFFECTS THE ACQUISITION OF HBV IMMUNITY AFTER UNRELATED-DONOR HEMATOPOIETIC STEM CELL TRANSPLANTATION

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**Introduction:** Patients undergone allogeneic hematopoietic stem cell transplantation (HSCT) are accompanied by intensive chemotherapy and immunosuppression. Such patients are in high risk of hepatitis B virus (HBV) infection. Recovery of recipient immunity on HBV post-transplantation has a potential role in HBV prevention. In the current study, we performed a retrospective analysis of recipient immunity on HBV after HSCT and attempted to find out some factors associated with the HBV immunity acquisition posttransplantation.
**Patients and Methods:** Between June 1999 and March 2009, 135 HBsAg(-) patients undergone unrelated HSCT were enrolled. HBV serum markers including HBsAg, HBeAg, HBeAb, HBsAb and HBcAb were tested for donor screen and recipients. HBV immunity was defined as positive HBsAb within 6 months posttransplantation. Patients were divided into 4 groups (R+/D+, R+/D-, R-/D+ and R-/D-; R represents recipient and D represents donor) according to + or - HBsAb in recipients and donors. The data were analyzed using spss v17.0 software.
**Results:** Pretransplantation characteristics of patients and donors were summarized in Table 1. Multivariate analysis showed that primary disease type, donor and recipient's sex and age, HLA disparity and conditioning regimen containing ATG were not statistically different among the 4 groups. In total 42 of 42 (100%), 22 of 32 (68.8%), 24 of 26 (92.3%) and 0 of 35 (0%) patients acquired HBV immunity posttransplantation in D+/R+, D+/R-, D-/R+ and D-/R- group respectively ($p<0.05$ between D+/R+ and D+/R- group, D+/R+ and D-/R- group, D+/R- and D-/R+ group, D+/R- and D-/R- group, D-/R+ and D-/R- group) indicating HBsAb in both donors and recipients had a significant favorable effect on maintaining the HBV immunity of recipients with HBsAb before transplantation. A total of 2 recipients experienced HBV reactivation posttransplantation(1 in D-/R+ and D-/R- group each) indicating HBV immunity in donors have protective effects on HBV reactivation. The mean duration from stem cell transfusion to HBsAb appearance was 25, 28, 20 and 24 days in the correspoinding group respectively ($p>0.05$).
**Conclusion:** Our data support that donor and recipient HBV immunity before transplantation can simultaneously affect the acquisition of HBV immunity and have a vital role in HBV reactivation after unrelated-donor HSCT. HBV vaccination should be performed in both donors and recipients who have no HBV immunity before transplantation.

## 409

### STREAMLINED ANALYSIS, QUANTIFICATION AND LONG TERM MONITORING OF POST-BONE MARROW TRANSPLANT CHIMERISM STR DATA

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Over the last decade, hematopoietic stem cell transplantation (HSCT) has become the main treatment for blood related and autoimmune diseases. After HSCT, chimerism monitoring plays a critical role in predicting the effects of engraftment, GVHD and disease relapse. We are introducing ChimerMarker® software which integrates raw data analysis to allele identification, chimerism quantification, reporting, and longitudinal monitoring. This program eliminates the need for manual data transfer from genotyping software to chimerism analysis software and will automatically identify informative loci using a patient specific chimertyping panel. After identification, percent chimerism and statistics for reliability assessment of each informative locus are calculated. Average chimerism is calculated for all informative loci that pass reliability assessment. Coefficient of variation, standard deviation, margin of error and number of informative loci are reported. Results can be printed. All samples and chimertyping panel can be saved within one project file. Subsequent samples for a particular patient can be easily appended to the project file and quantified within seconds. By having all test samples for one patient in one file, the software can construct a longitudinal graph for long term monitoring of chimeric status. This graph may be used to anticipate changes in the chimerism state that may warrant drug intervention to prevent GVHD or disease relapse. ChimerMarker software is superior to the current method of using spreadsheets. It eliminates error prone data transfer, time consuming manual loci identification, repetitive calculations, and allows for electronic record keeping of all samples, calculations, and longitudinal graph in one project file. This study uses data collected from a five-year post-bone marrow transplant patient. DNA was amplified using Profiler Plus®. Analysis, including: allele calls, determining informative loci, calculating percent chimerism and quality control metrics, and monitoring report graphing, was completed in ChimerMarker in approximately 30 minutes.

## 410

### UTILIZATION OF NURSE PRACTITIONERS IN BONE MARROW HARVESTS

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Over 100 bone marrow transplants are performed at Cincinnati Children's Hospital Medical center each year. Those recipients fortunate enough to have a matched related donor number from ten to twelve yearly. These donors are harvested at our facility through the efforts of our harvest team comprised of two physicians and a nurse practitioner. Bone marrow harvests require meticulous attention to detail during the collection and post when the marrow is filtered and transported to a processing center in preparation for infusion into the recipient. Specifics about how the nurse practitioner functions as a key member of the harvest team are outlined.

**Table 1. Pretransplantation Characteristics of Patients and donors before Transplantation**

| | D+/R+ group | D+/− group | D−/R+ group | D−/R− group | p value |
|---|---|---|---|---|---|
| Median recipient age(years) | 25 | 27 | 28 | 27 | >0.05 |
| Male recipient number | 27 | 20 | 16 | 21 | >0.05 |
| Conditioning regimen containing ATG | 5 | 3 | 4 | 3 | >0.05 |
| Donor mean age | 30 | 28 | 29 | 31 | >0.05 |
| HLA mismatched | 5 | 3 | 4 | 3 | >0.05 |
| Positive HBcAb in recipients | 12 | 8 | 7 | 9 | >0.05 |
| Total number | 42 | 32 | 26 | 35 | |